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Successful Transplantation of Hearts Harvested 30 Minutes After Death From Exsanguination

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The donor pool for heart transplants is severely limited. Unfortunately, many trauma patients who might be donors die of exsanguination before their organs can be used. We tested whether hearts "dead" for one half hour after exsanguination could be used as heart transplants in 8 lambs (mean weight, 8 kg). Four lambs were exsanguinated by severing the subclavian artery while simultaneously infusing intravenous saline solution to mimic resuscitation attempts. All animals died. Thirty minutes after hypotensive arrest and death, simulating the time needed to secure donation permission, the heart was harvested, perfused with 250 mL of cold cardioplegia containing 200,000 units of streptokinase to dissolve intravascular clots, and stored in iced saline solution for a mean of 1.5 hours while 4 recipient lambs were prepared for operation. After bypass and recipient heart excision, the "dead" donor heart was transplanted orthotopically. The heart was reperfused with low flow (25 mL/min), low pressure (30 mm Hg), low hematocrit (hematocrit, 0.08 to 0.12) blood supplemented with prostaglandin E_1 and nifedipine for 15 minutes, followed by full flow rewarming for 45 minutes. All hearts resumed normal contractions. All animals were weaned from bypass without inotropes. Pressures a half hour after bypass were (in mm Hg): aorta, 80 ± 10; pulmonary artery, 20 ± 5; right atrium, 9 ± 5; and left atrium, 9 ± 2. We conclude that hearts "dead" for one half hour after exsanguination are capable of being reanimated and used successfully as donor organs. With further development, this method could potentially greatly expand the donor heart pool.

(Ann Thorac Surg 1992;53:772–5)

The donor pool of brain-dead patients is the principal rate-limiting step that prevents more widespread application of heart transplantation. It is estimated that, at most, only one half of potential donors are utilized due to problems with consent or failure to meet established criteria for acceptability by a particular harvest team. More importantly, there exists a much larger potential donor pool that consists principally of young adults who have died suddenly secondary to exsanguination from penetrating or blunt trauma. These victims, who prior to their precipitous demise had normal organs, might be used as donors, after obtaining appropriate consent for donation, if methods could be devised to reliably resuscitate their individual organs.

Encouraged by our initial success in "reanimating" hypoxically arrested, asystolic hearts in a lamb model, we elected to apply these concepts to a traumatic exsanguination model where factors such as intravascular coagulation and time intervals to secure permission for organ harvest might limit the concept's usefulness [1]. This report details the preliminary findings of our experimental preparation.

Presented at the Twenty-seventh Annual Meeting of The Society of Thoracic Surgeons, San Francisco, CA, Feb 18–20, 1991.

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Material and Methods

Eight farm-raised juvenile lambs of 6 to 9 kg (mean, 8 kg) were selected as random pairs; where a size discrepancy existed, the larger of the 2 animals was chosen as the donor (Fig 1). Thus, 4 animals were to become donors, whereas the other 4 served as recipients.

The donor lambs were anesthetized with ketamine, intubated, placed on a Servo C volume ventilator and maintained on halothane anesthesia. Sixteen-gauge intravascular catheters were placed in the femoral vessels, and the arterial pressure was transduced. A median sternotomy was performed, and the right subclavian artery was dissected and looped. After stabilization, the artery was partially transected, resulting in exsanguination over a 2- to 5-minute period. Simultaneously, 500 mL of normal saline solution was administered intravenously through the femoral vein catheter to stimulate a resuscitation attempt.

Despite this fluid resuscitation, blood pressure dropped progressively over a 3- to 5-minute period until it became unobtainable. Cardiac electrical activity usually showed initial tachycardia followed by progressive bradycardia leading to cardiac standstill 5 to 14 minutes after zero blood pressure. The animal was left undisturbed for a further 30 minutes. Rectal temperatures averaged 39°C.

After the 30-minute period, simulating the time needed to secure organ donation permission, the pericardium was opened, the ascending aorta clamped, and the heart perfused with 250 mL of 4°C Roe's solution to which was

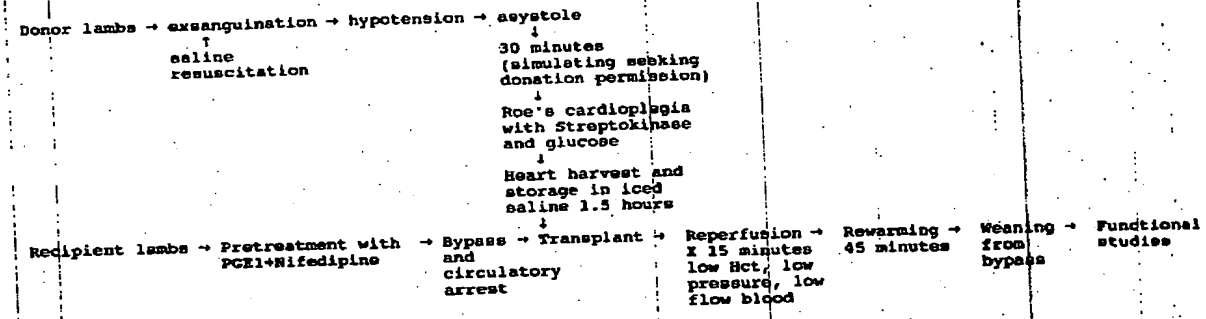

Fig 1. Experimental protocol. (Hct = *hematocrit*; PGE1 = *prostaglandin* E_1.)

added 200,000 units of streptokinase and 10 mL of 50% dextrose. The heart was then harvested, inspected for any gross ventricular cavity thrombi, and placed in iced saline solution for a mean of 1.5 hours.

After donor harvest, recipient lambs were similarly anesthetized and cannulated, and a median sternotomy was performed. The pericardium was incised and fashioned into a cradle. The animals were heparinized with 4 mg/kg of sodium heparin. The ascending aorta was cannulated through a pursestring for arterial inflow and the right atrial appendage cannulated for venous egress to the pump-oxygenator. The animals were placed on bypass using an asanguineous prime and cooled to a rectal temperature of 20°C.

During bypass, prostaglandin E_1 administration was started at 0.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ and continued until 30 minutes before weaning from bypass. Simultaneously, a 5-mg nifedipine capsule was opened and its contents were inserted sublingually.

After cooling, the ascending aorta was cross-clamped and profound hypothermic arrest instituted. Cardiac transplantation was performed in our usual fashion, using the "dead" donor heart after excising the recipient's heart [2]. The venous cannula was reinserted into the donor's right atrium, the left atrial appendage was vented, and the lamb was placed back on bypass and rewarmed. With the aortic cross-clamp still in place, an 18-gauge cannula was inserted into the ascending aorta and connected to a separate line from the pump oxygenator, which delivered low hematocrit (0.08 to 0.12), low Ca^{2+} (ionized calcium, 0.3 to 0.5 ng/dL), warm blood at low pressure (30 mm Hg) and low flow (25 mL/min) for 15 minutes. The cross-clamp was then released, the hematocrit concentrated, and calcium repleted during a further 45-minute rewarming period. Bradycardia or temporary heart block was treated with boluses of 10 μg of isoproterenol, but none was given after cessation of bypass. All four hearts returned to sinus rhythm before weaning from bypass.

Results

After a total reperfusion period of 1 hour, all four hearts were weaned from bypass. Pressures were measured with indwelling needles at 1, 5, 15, and 30 minutes after bypass. At 30 minutes after bypass systolic aortic pressure was 80 ± 10 mm Hg, pulmonary artery pressure was 20 ± 5 mm Hg, and right and left atrial mean pressures were 9 ± 5 and 9 ± 2 mm Hg, respectively. Hemodynamics remained stable throughout the observation period, and no animal's condition deteriorated before termination of the experiment. All animals were then euthanized and their hearts recovered.

Comment

In recent years, efforts by Buckberg and his colleagues have suggested that cardiac ischemia of prolonged duration does not, in and of itself, lead to cardiac necrosis. In the past, irreversible cardiac muscle damage has been judged to occur after ischemia based upon pathologic studies of myocytes examined after myocardial blood flow restoration, presupposing that restoration of blood flow did not impart a reperfusion injury of its own [3]. Thus, periods of more than 15 minutes of warm ischemia have been thought to produce degrees of irreversible myocardial damage or necrosis, characterized pathologically by contraction bands and calcium deposition within swollen, deranged mitochondria [4]. Although little doubt exists that these changes do indeed occur with the restoration of normal arterial blood flow, it is now equally apparent that the restoration of such "normal" blood flow may be wholly or partially responsible for this "ischemic" necrotic damage via numerous mechanisms of reperfusion injury.

Altering the extent of reperfusion injury in the globally ischemic heart has been the subject of intensive laboratory and clinical work by ourselves and others in recent years. Our laboratory effort parallels our clinical findings that donor hearts obtained from infants resuscitated from sudden infant death syndrome, often after prolonged "down" times of 20 to 40 minutes, function well both in the short and medium term after being transplanted [5]. These clinical findings mirror our initial experimental results, that neonatal lamb hearts subjected to hypoxic arrest can be successfully transplanted and reanimated after 10 to 21 minutes of warm asystole [1]. The donors in these previous experiments were pretreated with prostaglandin and nifedipine, so that the present experiment

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adds additional potential risks of ischemic injury and reperfusion injury in an unpretreated donor.

The methods chosen for reperfusion in this model are based on easily controlled variables, which can be altered in a traditional bypass circuit. Specifically, (1) the use of a low-calcium cardioplegic agent such as Roe's solution may lessen the amount of extracellular calcium available for calcium paradox [6] and (2) similarly, the use of an asanguineous prime, with its resultant hemodilution, dramatically reduces ionized calcium levels in the reperfusate. Low calcium reperfusion alone significantly reduces reperfusion injury but (3) coupling this with a calcium channel blocker such as diltiazem further prevents calcium paradox within the myocardium [7]. (4) Addition of high amounts of dextrose to the cardioplegia solution or donor's circulation, or both, has been a mainstay of our clinical transplant program [2] and has been shown by Okamoto and his co-workers [8] to significantly improve myocardial function when included in cardioplegia solutions. (5) Controlled reperfusion with low-pressure, low-flow blood has been shown to significantly salvage myocardial contractility after prolonged ischemia, particularly if continued for more than 10 minutes [9, 10], and can be easily administered via any roller pump during initial rewarming. The mechanisms for this beneficial effect are unclear, but low-flow/low-pressure reperfusion most likely prevents myocardial edema and the washout out of "rounded" endothelial cells within the vasculature, allowing smooth muscle relaxing factor to continue to be produced, limiting the "no-reflow" phenomenon.

It is possible that other combinations of agents and maneuvers may produce better or at least equal effects on the recovery of severely ischemic myocardium. Indeed, the recovery of hearts after 30 minutes of warm ischemia while on cardiopulmonary bypass is possible, but represents an entirely different set of circumstances than described here. It was not surprising to us then, that in preliminary, unpublished experiments, we could not resuscitate adult goat hearts when nifedipine and prostaglandin were not given during reperfusion. In fact, the use of prostaglandin in this study stems directly from our unpublished clinical observation of improved myocardial performance and overall clinical condition in infants who received prostaglandin E_1 during and after transplantation. Its role as a vasodilator in preventing the no-reflow phenomenon is certainly additive to that of the other agents described above.

Finally, it must be noted that these results are preliminary and use temporary survival as the only end point of success. Numerous other studies are required to delineate whether this phenomenon occurs in other animals and in what ages. But ultimately, one must answer the question as to how long is "too long" before cell death will occur regardless of the methods of reperfusion. Our findings suggest that traditional concepts of cell death require extensive reexamination; such reexamination may eventually lead to the conclusion that cadaveric hearts can be used successfully in transplantation.

In conclusion, we have demonstrated in an experimental lamb model that hearts that have been "dead" for one half hour after death from exsanguination are capable of reanimation and can be used successfully for donor organs. Although these findings are preliminary, further refinements could result in the utilization of an entirely new source of organ donors, which are readily available and extremely common.

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DISCUSSION

DR JOHN C. BALDWIN (New Haven, CT): Not surprisingly from such an innovative group as the Loma Linda group, this is a provocative and interesting report which addresses, as they point out, a very important problem. As we all know, there are approximately 50,000 people who die every year of congestive heart failure and who might be considered as transplant candidates. Although xenografting may be the ultimate answer to biologic replacement, this certainly might be an important other

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line of pursuit. This, as Dr Gundry pointed out, is not an entirely new idea. There has been interest in this phenomenon, particularly in Japan where there has been difficulty with the concept of brain death and where there have been some reports of attempts of cadaveric resuscitation of potential heart grafts, particularly after Dr Wada's singular experience with an attempted heart transplantation in Japan.

I think Dr Gundry and associates have quite properly focused on the reperfusion injury as the most important aspect of this model, as one is not particularly surprised by the absence of structural damage, per se. I think that the study would have been enhanced, however, by inclusion of some biochemical data as well as some assessment of myocardial edema, which certainly has been seen in longer preservation standard heart transplantation.

I think that those of us who worked in Palo Alto with Dr Shumway when no one else was doing heart transplantations in significant numbers may be responsible for excessive conservatism in evaluating donors, and these criteria, of course, have crept into textbooks and federal documents and so on. One of the questions I would like to ask Dr Gundry is whether or not their experience with this model has in any way influenced their attitude toward hemodynamic instability or toward the issue of brief periods of cardiac arrest, which have been, of course, accepted but really limited to brief defibrillations.

Second, I believe that it is important to ask why the saline resuscitation was administered. This may have had a significant flushing effect. It is unclear, of course, as to whether or not there were any clots dissolved by the streptokinase that was given, but the saline administration may be an important aspect of this model and one that would not always be duplicated in dealing with trauma victims.

Finally, I would ask whether he can provide us with a rationale for the use of the prostaglandin E_1 pretreatment in the recipient and also apparently during the reperfusion of the heart.

DR GUNDRY: I would like to thank Dr Baldwin for those very excellent comments. First of all, this study did not evaluate any biochemical changes within the heart or the presence or absence of myocardial edema. This study is a preliminary study, and (at least in our laboratory) our feelings have always been to try and keep it simple. Survivability without inotropes, even for a short period of time, was our end point and certainly justifies continuing this study as to the exact biochemical changes that are occurring in these hearts.

In regard to whether this particular study and other studies that we are doing in our laboratory have changed what we do with our donor hearts, that is absolutely true. Actually both have been synergistic in a way. We have found in our infant transplantation experience that infants who die of sudden infant death syndrome, who account for a great number of our donors, have had anywhere from 10 minutes to 40 minutes of documented downtime, during which no resuscitation attempts were undertaken, and may have had up to 2 hours of cardiopulmonary resuscitation by experienced people before their hearts resumed functioning. Now, unfortunately all of these children eventually succumb to brain death, but the amazing thing that we found was that their hearts have been perfectly good! These hearts have been restudied on long-term follow-up for more than 5 years now, and we have found no detrimental changes in their long-term myocardial function. Indeed, as a result of these studies we have been prepared to take donors who are in fact moribund. Many of our donors, thankfully, have been refused by four or five other teams. We will frequently take donors on very high-dose inotropes with very little blood pressure, and the results have been very satisfactory.

Finally, in regard to the saline resuscitation, we made every attempt in this model to mimic a clinical model. The problem with intravascular thrombosis is a very real one in the lamb model. You heard yesterday in a canine model that up to 2 hours without heparin in a dead dog does not result in significant intravascular thrombosis within the lung. We did not find that to be true in the lamb. In fact, the lamb does have extensive intravascular thrombosis. The saline resuscitation, however, was attempted to mimic our observations that most of the patients in the emergency room resuscitation setting have had a saline resuscitation attempt or some other form of crystalloid infusion. In fact, most of these patients do have inherent anticoagulation from their resuscitation attempts, which is clearly evident in the operating room. So whether this does affect the application of this technique to a patient who arrives without a saline resuscitation will have to be shown by further results.

The Language of BIOTECHNOLOGY
A DICTIONARY OF TERMS
SECOND EDITION

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thin-layer chromatography (TLC) \'thin-ˌlā-ər ˌkrō-mə-'täg-rə-fē ('tē 'el 'sē)\ A chromatographic separation developed from paper chromatography in which the stationary phase is a thin layer of a powdered adsorbent, usually silica or alumina, supported by a glass or aluminum sheet. Such plates are commercially available in a number of forms depending on the nature of the solid phase and type of binder. The chromatogram is developed by placing the plate spotted with the analyte mixture in a small amount of the mobile phase, which is usually an organic solvent or mixture of solvents. The mobile phase rises up the vertical plate by capillary action, and separation of the components occurs by partition between the solid support material and the mobile phase. After developing and drying the plate, the spots may be visualized in a number of ways selective for the type of compound or submitted to UV radiation, under which the organic compounds often fluoresce.

High-performance thin-layer chromatography (HPTLC) is a development of TLC that uses specialized adsorbent phases bonded onto the backing plate. Various adsorbents are commercially available for affinity chromatography or reversed-phase chromatography. High performance is also achieved by having an adsorbent of a regular particle size.

Thiobacillus ferrooxidans \ˌthī-ō-bə-'sil-əs ˌfer-ō-'äks-i-danz\ *See* microbial leaching.

thiol proteases \ˌthī-ˌól 'prōt-ē-ˌās-ez\ One of the four possible classifications of proteases. Thiol proteases have a thiol (—SH) group at the active site that is essential for activity. *See also* bromelain, ficin, papain.

thixotrophy \thik-'sä-trə-fē\ A time-dependent reversible behavior of a fluid under an applied shear stress, such that on application of a shearing force the fluid becomes less viscous. When the shear force is removed, the viscosity returns to its original value.

thixotropic fluid \ˌthik-sə-'trō-pik 'flü-əd\ A fluid that, when subjected to a constant shear stress, such as agitation, exhibits a reduction in apparent viscosity with time.

thrombin (E.C. 3.4.21.5) \'thräm-bən\ A blood coagulation enzyme responsible for the conversion of soluble fibrinogen to insoluble fibrin, which forms part of blood clots. Thrombin is a serine protease and is present in the serum as the inactive form prothrombin. Prothrombin is converted into thrombin as a result of tissue damage. *See also* fibrin, hirudin.

thrombolytic \ˌthräm-bə-'lit-ik\ Any compound that initiates dissolution of a blood clot (*thrombus*). *See*, for example, eminase, plasmin, streptokinase, tissue plasminogen activator, urokinase.

thrombus \'thräm-bəs\ *See* thrombolytic.

thylakoids \'thī-lə-ˌkȯidz\ Membranous structures, shaped like flattened sacs, found in chloroplasts. A pile of these sacs is a *granum*. The thylakoid membranes contain the chlorophyll molecules and other components of the energy-transducing machinery necessary for photosynthesis.

thymidine kinase (tk) gene \'thī-mə-dēn 'kī-ˌnās ('tē 'kā) 'jēn\ A widely used selectable marker for transfection studies in eukaryotic cells. *See* selectable marker.

ti (tumor-inducing) plasmid \'tē 'ī ('t(y)ü-mər-in-'d(y)üs-iŋ) 'plaz-məd\ *See Agrobacterium tumefaciens.*